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Impedimetric paper-based biosensor for the detection of bacterial contamination in water

Saravanan Rengaraj,^{1†} Álvaro Cruz-Izquierdo,^{2††} Janet L. Scott,² Mirella Di Lorenzo^{1*}

¹Centre for Biosensors, Bioelectronics and Biodevices and Department of Chemical Engineering, University of Bath, Bath BA2 7AY, United Kingdom

²Centre for Sustainable Chemical Technologies and Department of Chemistry, University of Bath, Bath, BA2 7AY, United Kingdom

* Corresponding author: m.di.lorenzo@bath.ac.uk

† Current address: Environment Centre Wales, School of Environment, Natural Resources and Geography Bangor University, Bangor, LL57 2UW, UK

†† Current address: Institute of Process Research and Development, School of Chemistry, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, United Kingdom

Abstract

According to the World Health Organisation, worldwide waterborne diseases are responsible for nearly two million human deaths annually. Rapid and at-site screen of pathogenic microorganisms in drinking water can help to markedly reduce this number. Here we report an innovative, simple and low-cost, paper-based probe for detection of bacteria in water, fabricated by screen printing carbon electrodes onto hydrophobic paper. Electrochemical characterization of the printed electrodes confirmed fast-electron transfer, with an estimated electroactive surface area of 0.25 cm^2 . The electrode surface was functionalised with carboxyl groups, prior to covalent immobilisation of the lectin *concanavalin A* (Con A), used as the biorecognition element. The system was then tested as an impedimetric sensor for bacteria in water. A linear increase in the probe charge transfer resistance was observed for bacterial concentrations ranging from 10^3 to 10^6 CFU mL^{-1} , with an estimated lower detection limit of $1.9 \times 10^3 \text{ CFU mL}^{-1}$. Considering its remarkable simplicity, cost-effectiveness and biodegradability, the sensor here reported could be an attractive solution for portable testing kits that address the challenges of traditional time-consuming and expensive lab-based analyses.

Keywords – Screen printed electrode, Paper electronics, Electrochemical impedance spectroscopy, Lectin-bacteria complex

1. Introduction

Water contaminated by microbial pathogens can lead to waterborne diseases, such as cholera, dysentery, typhoid, and polio, which have been defined as the world's leading killers by the World Health Organisation. The mortality incidence from waterborne diseases is higher in remote and poor parts of Africa and Asia, where basic sanitation systems are often lacking. In these areas, strict controls of drinking water resources can be extremely challenging. Traditionally, this monitoring requires time-consuming analyses, performed by skilled personnel in laboratories equipped with instrumentation of prohibitive cost for poor countries. The development of point-of-need detection tools, which are rapid, low-cost and easily deployed by non-expert users can play an important role in preventing and/or minimising waterborne diseases. [1]

Electrochemical-based biosensing technologies hold great promises for on-site and real-time monitoring. [2-4] These biosensors can be classified according to the nature of the biomolecular interactions, which include antibody/antigen, [5] enzymatic, [6] and DNA-DNA interactions, [7] as well as detection of cellular structures or cells. [8] Impedimetric biosensors (IB), in particular, have gained considerable attention in bioanalytical methods because of the ease of signal quantification, convenient miniaturization, and integration into low cost assays. [9] The main advantage of such systems is the ion label-free detection of the target analyte, *sans* fluorescent dyes, enzymes, or radioactive labels. By coupling a bio-recognition element to an electrode, a wide range of analytes, from proteins to bacterial cells and viruses, can be detected with detection times as low as a few minutes. [10]

Recently, paper-based electronics have gained much attention as an effective way to develop miniature, light and, eventually compostable, devices at low cost. [11] Conductive ink can be screen printed onto paper substrates and designs and structures are readily tailored according to the target end use. [12] Printed electrodes on paper have already been used for electrochemical, [13] electrochemiluminescence, [14] and photoelectrochemical [15] detection systems. Paper-based sensing platforms have been suggested for dipstick tests, [16] microfluidic paper-analytical devices (μ PAD), [14] and lab-on-chip [17] devices, and successfully used for the detection of a range of analytes, such as glucose, uric acid, and cancer biomarkers. [18-20]

Paper-based sensors for detection of bacteria in food and water samples have also been reported. [21, 22] Most of these devices use colorimetric detection system, [23-25] although

systems for immunoassay impedance measurements have also been suggested. [26] Colorimetric assays, which are based on the interaction between the bacteria and a biorecognition element (usually an enzyme) leading to a chromogenic product, can suffer from poor selectivity and difficulties in performing quantitative measurements. [27] Immunoassay detection systems, which use antibodies as bioreceptors, are associated with low capture efficiency, instability due to environmental fluctuations, high cost of production, and lengthy analysis times, which can limit their field applicability. [28]

To address these challenges, in this study we have developed innovative functionalised paper-based electrodes for impedimetric detection of bacteria in water. In particular, the sensing probe was fabricated by screen printing a conductive carbon ink onto a commercial hydrophobic paper. *Concanavalin A* (ConA) was chosen as the biorecognition element, due to its ability to selectively interact with mono- and oligo-saccharides in bacterial cells. [29, 30] The electrochemical performance of the resulting sensor was investigated, and its ability to detect bacteria in water samples tested. For comparison, commercial pyrolytic graphite electrodes (PG), functionalised in the same manner, were also tested. To the best of our knowledge, this is the first time that the hydrophobic properties of a paper substrate have been coupled with the electrochemical functionalization of the electrodes to demonstrate rapid, label-free, impedimetric bacterial detection.

2. Experimental

2.1. Materials.

Potassium dichromate, ethanolamine hydrochloride, *N*-ethyl-*N*-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and lectin (*Concavalin A*), were purchased from Sigma–Aldrich. All reagents and buffer solutions were prepared with deionized water (Millipore-Q, 18.2 M Ω cm). Pyrolytic graphite electrodes (PG) were purchased from IJ Cambria, UK. Carbon/graphite conductive ink (C2130307D1) was purchased from Gwent Group (Cardiff UK).

2.2. Bacteria culture and growth conditions

Bacterial cultures from sewage sludge (Wessex Water Scientific Laboratory, Saltford, Bristol) were grown overnight at 37 °C in a synthetic wastewater. The synthetic wastewater contained: 2 mM (NH₄)₂SO₄, 0.2 mM MgSO₄·7H₂O, 0.03 mM MnSO₄·H₂O, 1.5 mM NaHCO₃,

0.01mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.03 mM $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$ and 100 mM $\text{CH}_3\text{CO}_2\text{K}$, used as the carbon source for the bacteria. After overnight growth, the cultures were filtered through 100 μm filter paper to retain large suspended solids, while allowing bacteria to pass through. The viable colonies were enumerated by the microbial plate count method and found to be approximately 10^8 CFU mL^{-1} in the overnight culture, defined as the stock solution. Samples with variable bacterial concentrations, were obtained by serial dilution of this stock solution in sterile phosphate buffer solution (PBS, 0.1 M, pH 7.4).

2.3. Screen printed electrode fabrication and characterization

The paper-based probe was fabricated by screen printing three layers of a carbon-based conductive ink onto Fabriano 5 HP paper. The three layer printing methodology was selected as the best compromise between low resistance and rapid printing (i.e. minimum number of layers). The resistance measured scaled as: $40 \Omega \text{ cm}^{-2}$ (one layer), $25 \Omega \text{ cm}^{-2}$ (two layers) and $18 \Omega \text{ cm}^{-2}$ with three printed ink layers. The addition of further layers did not significantly reduce the resistance further. Each ink layer was dried at 60°C for 30 min and the final device consisted of a circular working electrode (6 mm diameter), with a geometric surface area of 0.286 cm^2 , printed onto a paper strip 4 cm long and 1 cm wide. The hydrophobicity of Fabriano 5 HP paper was measured using the sessile droplet method, with 1.6 μL DI water droplets, using a contact angle system OCA 25 (Dataphysics Instrument, UK). The water contact angle (CA) measurements were recorded five minutes after the droplet was dispensed with a syringe. To cross-link the cellulose fibres, after screen printing the electrodes, the paper was submerged for 3 h in a solution of 6% w/v glyoxal at room temperature ($20 \pm 3^\circ\text{C}$), followed by a thermal treatment at 140°C for 1 h. The resistance of the electrodes was measured using a four-point probe method and a cyclic voltammetry sweep from 0.01 to -0.01 V, at a scan rate of 5 mV s^{-1} . This procedure helps to improve the tensile strength of the paper substrate, making the sensor more robust in use, and also enhances the electrical performance of the screen printed electrode [31].

2.4. Electrode surface modification

Lectin immobilization was achieved by electrochemically modifying the electrode surface. Briefly, the carbon electrodes, either a pyrolytic graphite electrode (PG) or screen printed carbon paper (SPC) electrode, were electrochemically oxidized by linear sweep voltammetry (LSV) at a scan rate of 5 mV s^{-1} , from 1.55 to 1.76 V vs. Ag/AgCl, in an aqueous electrolyte containing 2.5% $\text{K}_2\text{Cr}_2\text{O}_7$ and 10% HNO_3 . To activate the carboxylic groups, the electrodes were incubated with 20 μL of 1:1 freshly prepared EDC/NHS for 20 minutes at room

temperature. Afterwards, the electrodes were incubated with 10 μL of lectin (2 mg mL^{-1} in 0.1 M sodium acetate buffer, pH 4.5) for 30 minutes. Subsequently, unreacted sites were blocked by exposing the electrode surface to a 1 M ethanolamine hydrochloride solution for 20 minutes to prevent any non-specific adsorption. Figure 1a is a schematic illustrating each step of functionalisation of the carbon electrodes, while Figure 1b shows the overall screen-printed device and principle of operation. After each step of surface modification, the electrodes were carefully rinsed with deionized water. To avoid evaporation of solution during exposure to lectin, humidity was maintained by exposure to water soaked absorbent paper during the electrode functionalization procedure. The success of each functionalization step was verified by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

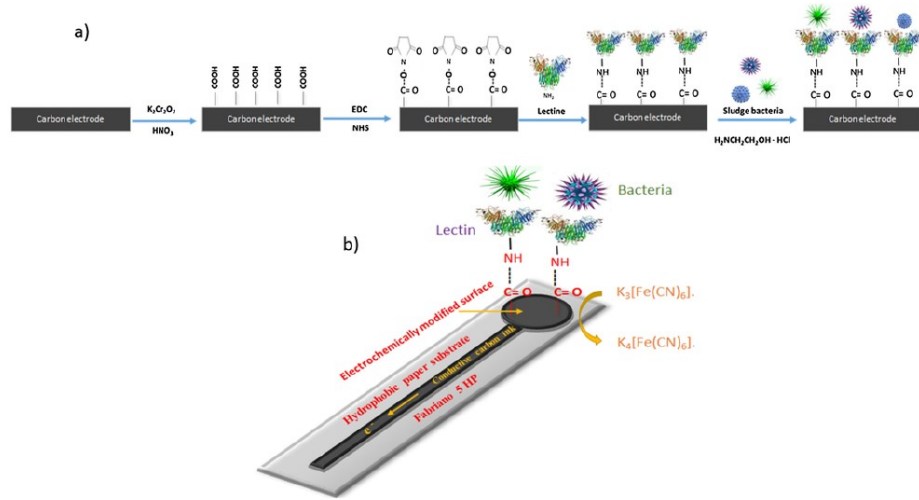


Fig. 1. (a) Schematic diagram showing the several steps of electrode surface modification and the principle of bacterial detection by the SPC biosensors. (b) Schematic representation of the use of the functionalised screen-printed probe for bacterial detection.

2.5. Electrochemical measurements

All the electrochemical measurements were performed using a potentiostat equipped with an impedance analyzer (Autolab PGSTAT and PalmSens3). A standard three electrode cell was used for electroanalysis, comprising of a working electrode, either PG or SPC, a reference electrode (Ag/AgCl) and a counter electrode (Pt wire). The electron diffusion coefficient was calculated using the Randles-Sèvcík equation (1). [32]

$$i_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} v^{1/2} C \quad (1)$$

where: i_p is the maximum current maximum (A); n is the number of electrons transferred in the redox event; A is the electroactive area of the working electrode (cm^2); D is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$), for ferricyanide it is estimated to be $2.2 \times 10^{-6} \text{ cm}^2 \text{s}^{-1}$; [33] C is the concentration of ferricyanide (mol cm^{-3}); and v is the scan rate (Vs^{-1})

All electrochemical measurements were performed in phosphate buffer at pH 7.4 in the presence of 5 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ as a redox probe. Cyclic voltammetry (CV) was carried out within the potential range -0.5 - +0.9 V vs. Ag/AgCl. The impedance spectra were obtained at 0.2 V vs. Ag/AgCl in a frequency range of 1 to 100 000 Hz, with a frequency modulation of 10 mV. The Randles circuit model used is shown on the insert of Figure 2a. It consists of an ohmic resistance (R_s), a double layer capacitance (C_{dl}), an electron-transfer resistance (R_{ct}) and a Warburg impedance (Z_w). R_s and Z_w represent the properties of the electrolyte solution, which affect resistance and diffusion of the redox probe. C_{dl} and R_{ct} depend on the dielectric, or insulating, features at the electrode-electrolyte interface, influenced by the change in the resistance occurring at the electrode interface. The impedance Z is reported as a Nyquist plot (Z_{im} vs. Z_{re}).

To investigate the response to several bacterial concentrations, the functionalised electrodes were exposed for 45 minutes to bacterial solutions obtained from serial dilution of the stock solution in a sterile phosphate buffer solution containing 0.5 mM of Mn^{2+} , Ca^{2+} , and Mg^{2+} . The lower limit of detection was estimated by considering the minimum concentration of bacteria that could be quantified with acceptable accuracy and precision. Experiments were conducted in duplicate.

2.6. AFM imaging of SPC electrodes

Tapping-mode AFM measurements were performed using a Bruker multimode Atomic-force microscope IIIa (Nanoscope), equipped with NuNano AFM probes (NuSENSE scout 350). The Nanoscope 6.14r1 software was used to analyze the topography of the samples, while the AFM images were processed with Bruker Nanoscope analysis 1.7 software.

3. Results and discussion

3.1. Investigation of electrode surface modification

A novel electrochemical one-step surface modification approach was developed to functionalise carbon electrode surfaces with carboxylic groups. The functionalisation strategy was first developed and tested on commercial pyrolytic graphite (PG) electrodes. PG electrodes were chosen because of their low background current, and ease of regeneration of the surface for repeated experiments. [34] The electrochemical oxidation of the carboxyl group, was achieved by LSV scanning at high potential, ranging from 1.55 to 1.76 V vs. Ag/AgCl (Figure S-1). The oxidative current appeared around +1.67 V. To monitor electrode surface

modification and changes on the PG surface, $\text{Fe}(\text{CN})_6^{3-/4-}$ was used as a redox system. EIS measurements were recorded at the formal redox potential of ferricyanide, 0.2 V vs. Ag/AgCl.

In Figure 2a the Nyquist plots obtained for bare and modified PG are compared. As expected, the bare PG electrode exhibited a fast electron transfer process. On the other hand, the electrode functionalized sequentially with COOH groups and lectin, exhibited increasing charge transfer resistance (R_{ct}) values. The R_{ct} was 0.58 k Ω and 3.87 k Ω for the COOH and lectin modified electrode respectively, compared to 0.095 k Ω for bare PG. The sequential electrode surface modification steps were also investigated by cyclic voltammetry in a 5 mM ferricyanide solution. As shown in Figure 2b, in the case of bare PG, a reversible redox couple was observed at 0.2 V vs. Ag/AgCl, while the COOH modified PG showed a decrease in peak current. The covalently attached lectin further decreased the peak current, with large peak-to-peak separation compared to peak currents of bare PG.

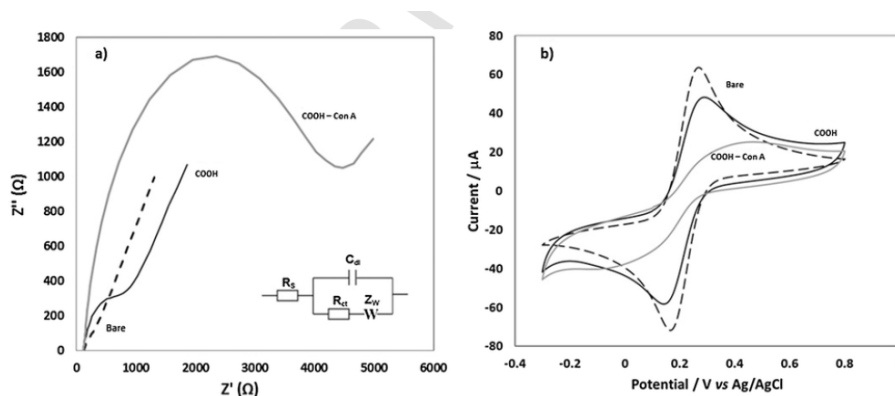


Fig. 2. Functionalization of the PG electrodes. a) Nyquist plots. The inset shows the Randles equivalent circuit for extraction of R_{ct} from impedance spectra. b) Cyclic voltammograms at a scan rate of 5 mV s⁻¹. In both graphs: the dashed line refers to bare PG; the black solid line refers to carboxyl functionalized PG; and the grey solid line refers to the electrode prepared by covalent immobilization of lectin Con A on activated PG. CVs and impedance measurements were performed in phosphate buffer (0.1 M) in the presence of 5 mM of $\text{Fe}(\text{CN})_6^{3-/4-}$ as redox probe.

Figure 3a shows the EIS response of the functionalised PG electrode at varying bacterial concentration, within the range $10^3 - 10^5$ CFU mL⁻¹. As shown, the R_{ct} increased with increasing bacterial concentration. It has been reported that Con A specifically binds with the mannose residues distributed on the surface of bacterial cells, such as *E.coli* and *Bacillus* sp. [35] These species are abundant in sewage and, therefore, were expected to be present in the sample tested in this study. [36] Lectin, however, might also interact with other types of bacteria in the sample, particularly if these present mannose residues on the surface of their cells. The increase in the R_{ct} is, therefore, a result of the formation of lectin-bacterial cell complexes on the electrode surface. As shown in Figure 3a, the R_{ct} values increased from 3.87 k Ω , for lectin modified PG electrodes in the absence of bacteria, to 5.85 k Ω , in the presence of

10^5 CFU mL⁻¹ bacterial cells. The charge transfer resistance increased proportionally with the logarithmic concentration of bacterial cells within the range of 10^3 - 10^5 CFU mL⁻¹, showing an **estimated** low detection limit of 3×10^3 CFU mL⁻¹ (Figure 3b). This result is in line with the work by Tolba *et al.* on impedimetric bacterial biosensors for detection of *Listeria* cells, where an increase in the charge transfer resistance was observed when endolysin modified electrodes captured *Listeria* cells. [37]

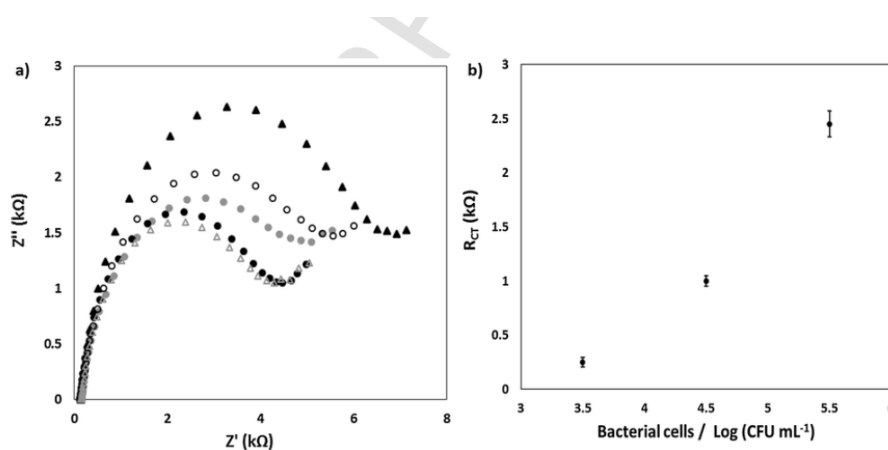


Fig. 3. Testing the use of the functionalised PG for detection of bacteria. a) Nyquist plots obtained at increasing bacterial concentrations, 10^3 CFU mL⁻¹ (●), 10^4 CFU mL⁻¹ (○), 10^5 CFU mL⁻¹ (▲), along with background impedance plots, obtained by testing the response of the lectin-modified electrode tested in fresh synthetic wastewater with no bacteria (●) and in synthetic wastewater incubated overnight at 37 °C with no bacteria (Δ). b) Charge transfer resistance response to varying bacterial concentrations.

3.2. Characterization of the SPC electrodes

Following the success with the PG electrode, screen-printed carbon (SPC) electrodes were fabricated, characterised, functionalised and then tested as sensor for bacteria detection.

SEM images of the SPC electrodes show a uniform coating of carbon ink (Figures 4a and b). The resulting electrode exhibited a four-point sheet resistance of $18.7 \pm 0.25 \Omega \text{ sq}^{-1}$. The paper substrate has a contact angle of 101.8° (Figure 4c), while the addition of carbon ink decreases this value to 71° (Figure 4d). The paper hydrophobicity is clearly a key feature, which facilitates the electrode functionalisation with COOH and lectin and allows practical applications of the resulting probe.

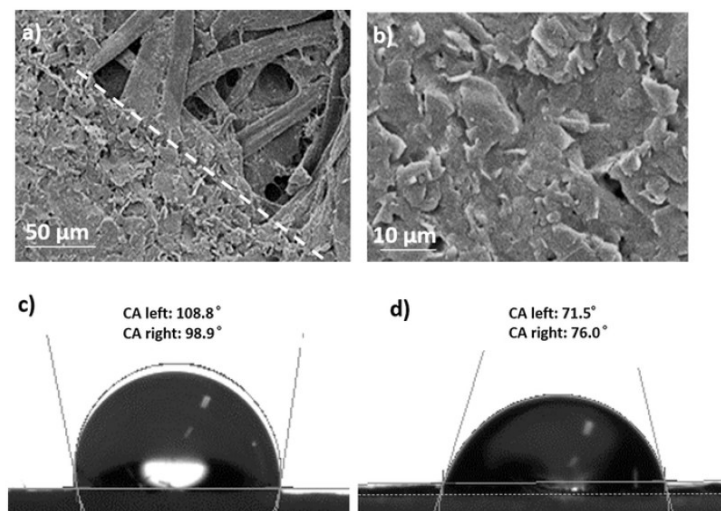


Fig. 4. SEM images of the paper surface printed with conductive carbon ink: (a) printed/non-printed interface, border indicated with the dashed line and (b) carbon ink coating. Contact angle of the Fabiano paper (c) blank and (d) of the paper after screen-printing the electrode.

The electrochemical performance of the screen-printed electrode was tested in ferricyanide by CV scans. As illustrated in Figure 5a, a well characterized and electrochemically reversible redox couple was observed at 0.18 V vs. Ag/AgCl, which corresponds to the ferri/ferrocyanide redox couple. The scan rate dependent study of SPC within the range 5 to 100 mV s^{-1} , is also reported. The peak current (i_p) versus square root of scan rate ($v^{1/2}$) was linear, suggesting a diffusion-controlled electron transfer process. Using the slope of scan rate dependent study, an active electrode surface of 0.25 cm^2 was estimated using the Randles-Sêvcík equation 1. [32]

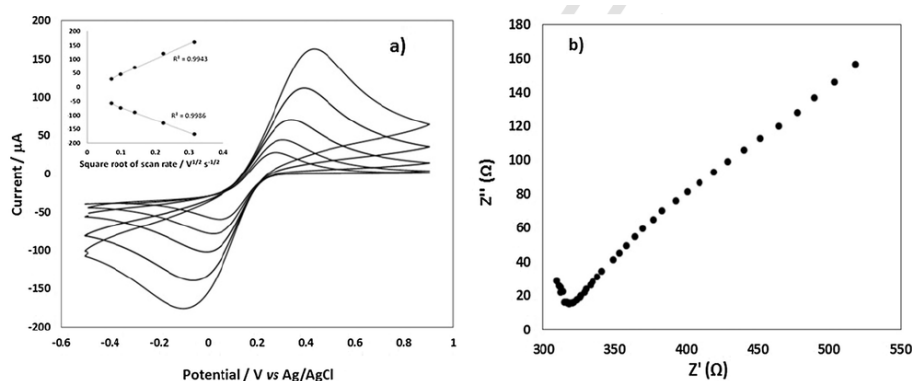


Fig. 5. Electrochemical characterization of the SPC electrode. a) Scan rate dependent study, at 5, 10, 20, 50 100 mV s^{-1} , in the presence of 5 mM $\text{Fe(CN)}_6^{3-/4-}$ in 0.1 M of PBS (pH 7.4). b) Electrochemical impedance spectroscopy of the bare SPC electrode.

EIS measurements were conducted to further verify the charge transfer behaviour of the paper electrode. As shown, the impedance spectrum is characterised by fast electron

propagation, with R_{ct} value of 420 Ω (Figure 5b).[38] The low R_{ct} with respect to the PG electrode can be attributed to the possible interference of the cellulose fibre network on electron diffusion. This is supported by SEM image that provides evidence of homogenous distribution of conductive carbon ink over the cellulose fibre network of the paper substrate (Figure 4). Similarly, Zang *et al* observed a low electron transfer resistance with their patterned 3D microfluidic paper-based analytical device (μ PAD) developed for electrochemical immunoassay. [38] To investigate the stability of the SPC electrodes, CV tests in 5 mM ferricyanide solution were performed over three days (Figure S-2). As shown, the electrochemical signal was stable during these days. In particular, the redox peak current measured on day 1 (67 μ A) remained unchanged over the increased peak current of day 3 (72 μ A), with an insignificant 70 mV shift in redox potential.

3.3. Analytical performance of SPC

Once the SPC was electrochemically characterised, the electrode surface was modified according to the methodology previously optimised for the PG electrode. Similarly, the functionalisation steps were monitored by both CV and EIS (Figure S-3 a & b). Also in this case, a shift in the redox couple compared to bare SPC is observed, which is a consequence of the covalent attachment of lectin. The EIS tests show an increase in the charge transfer resistances, from 420 Ω for the bare SPC, to 640 Ω and 872 Ω for the COOH and lectin-modified electrodes respectively.

The EIS response of the functionalised SPC electrodes to bacteria in water was subsequently tested (Figure 6a). As shown, the interfacial electron transfer resistance increased with increasing bacterial concentrations. No significant increase in impedance was observed with the COOH modified electrodes when exposed to large concentration of bacteria, thus demonstrating that detection is associated to the interaction between the bacteria cells and lectin only. A good correlation between the electron transfer resistance and the logarithmic value of cell concentration was found within the range 10^3 - 10^6 CFU mL⁻¹, with a coefficient $R^2 = 0.997$ (Figure 6b), and an **estimated lower** limit of detection of 1.9×10^3 CFU mL⁻¹. Above this range, the detection signal was saturated. The spectra obtained from the experimental data was fitted with the equivalent circuit to extract the R_{ct} value (Figure S-4). Overall, these results demonstrate the effectiveness of the surface functionalisation methodology developed, along with its versatility, proven by the ease of transferring it from expensive commercial pyrolytic graphite electrodes to cheap paper-based screen-printed electrodes.

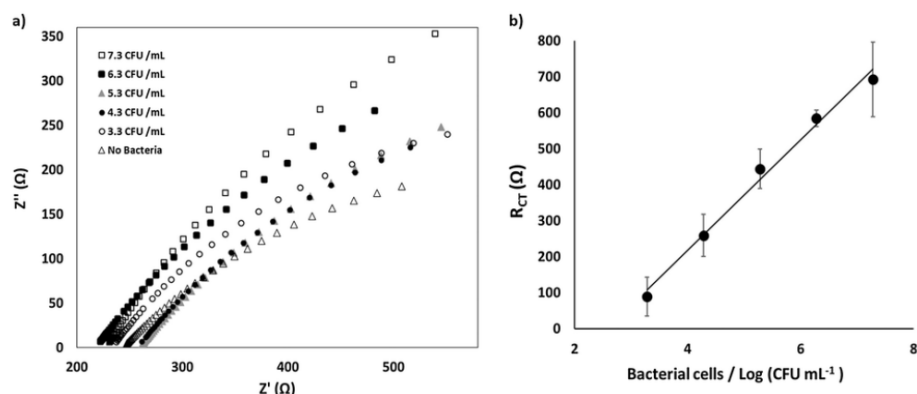


Fig. 6. Testing the use of the functionalised SPC electrodes for bacterial detection. a) Nyquist plots obtained with different concentration of bacterial cells. Impedance spectra were recorded at the formal redox potential $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.1 M of PBS (pH 7.4). b) Change in the charge transfer resistance, R_{ct} , as a function of the bacterial concentration. Data are obtained by subtracting the blank (R_{ct} of lectin modified electrode without bacteria) and are the average of duplicate measurements.

Figure 7 shows the AFM topography of the SPC electrode functionalised with lectin before (Figure 7a and b) and after (Figure 7c and d) exposure to a bacterial concentration of 10^7 CFU mL⁻¹. As shown, upon exposure to bacteria, rod-shaped and coccus-shaped bacteria are evident on the electrode surface. The different bacterial shapes are ascribed to the fact that a mixed community from sewage was tested.

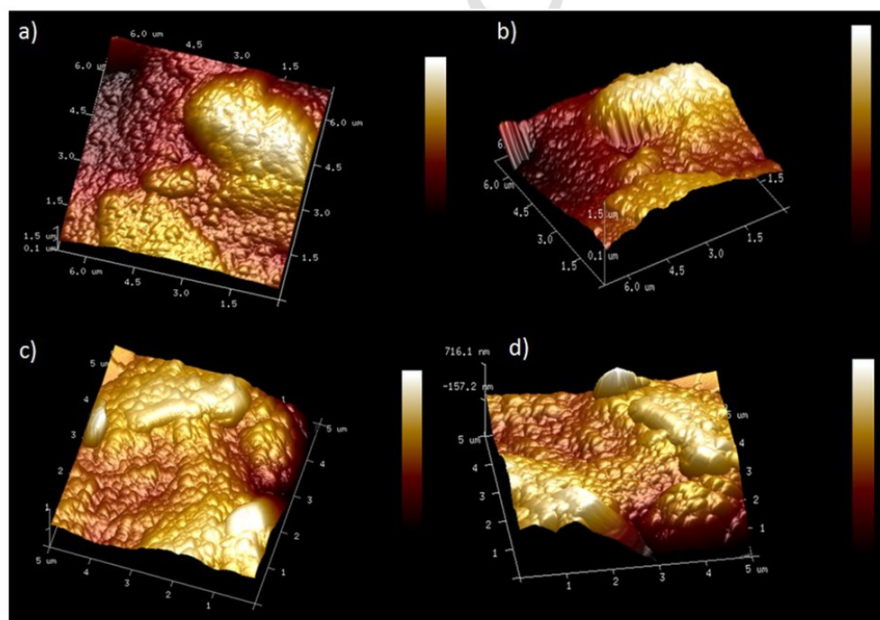


Fig. 7. Atomic force microscopy (AFM) topographic images of the SPC electrode functionalised with lectin, prior (a and b) and after (c and d) exposure to a solution containing 10^7 CFU mL⁻¹ of bacteria. The AFM images were tilted to better visualise the morphology of the lectin-bacterium complexes.

In Table 1 we summarise the results obtained with our SPC electrode and compare these with impedimetric biosensors previously reported, on the basis of electrode material and surface functionalization. The use of lectin as a biorecognition element for bacteria has been previously reported, although only on gold screen-printed electrodes. In particular, Gamella *et al.* reported gold screen printed electrodes functionalised with biotinylated lectin, for the impedimetric detection of *E.coli* with a detection limit of 5×10^3 CFU mL⁻¹, and a dynamic range of 5×10^3 to 5×10^7 CFU mL⁻¹. [35] Similar results were observed with biotinylated polyclonal anti-*E.coli* linked to a self-assembled monolayer modified gold electrode for detection of lysed *E.coli*. [39] Ruan *et al.*, reported an impedimetric immunosensor for *E.coli* detection using an enzyme-labelled signal amplification. [40] Wang *et al* reported a paper-based impedimetric immunosensor based on graphene oxide and gold nanoparticles. [26] Nonetheless, the detection limit obtained with our SPC electrode (1.9×10^3 CFU mL⁻¹) is lower than most impedimetric detection systems previously reported. [28, 35, 37, 40-42] It is also better than the limit achieved with surface plasmon resonance (SPR) lectin based biosensors previously reported. [43, 44] Moreover, our system has the advantage of cost-effectiveness compared to immunosensors, which are characterised by high production and purification costs and challenges associated with stability of the antibody used, or SPR sensors, which rely on expensive equipment not feasible for use in on-site analysis.

To further enhance the sensitivity and approach the threshold values for *E.coli* in water (10 - 1,000 CFU mL⁻¹), [45] follow-up work will focus on the use of nanostructured materials, such as carbon nanotubes. This approach would increase the electrode specific and active surface area of the SPC electrode, in turn improving the redox signal to achieve a lower detection limit. [11]

4. Conclusions

In this work, we report an innovative method for the functionalisation of carbon-based electrodes, which was easily transferred from commercial (and expensive) pyrolytic graphite electrodes to low-cost, easy-to manufacture and easy-to-dispose-of screen printed electrodes. The hydrophobicity of the paper used as a substrate facilitated the electrode functionalisation, preventing any unspecific adsorption of the several solutions used during the electrode preparation. The cellulose that was the bulk of the paper, upon which the device was constructed, was cross-linked prior to use to enhance the strength of the paper substrate and the

electrical performance of the screen-printed electrodes, which were characterised by very low R_{ct} values. Finally, we demonstrated the use of the so-functionalised electrodes for rapid detection of bacteria in water. The estimated bacterial detection limit obtained with the functionalised screen-printed electrodes was 1.9×10^3 CFU mL⁻¹, which is lower than other impedimetric sensors previously reported, and the dynamic range was $10^3 - 10^6$ CFU mL⁻¹.

The simple manufacturing process here reported, combined with the use of paper, guarantees the affordability of the SPC sensor as an alternative to expensive laboratory-based analyses.

Acknowledgements

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Table 1. Comparison of the SPC electrode performance with other impedimetric biosensors for bacterial detection.

Transducer	Mechanism of detection	Linear range (CFU mL ⁻¹)	Bacterial detection limit (CFU mL ⁻¹)	Reference
Screen-printed gold electrode	Biotinylated ConA– <i>E. coli</i> complex onto Au/SPE	$5 \times 10^3 - 5 \times 10^7$	5×10^3	[35]
Indium tin oxide (ITO) electrode chips	Antibodies immobilized over epoxysilane monolayer on ITO substrate for enzyme-labelled signal amplification	$6 \times 10^3 - 6 \times 10^7$	6×10^3	[40]
Indium tin oxide (ITO)	Chitosan doped (CS / reduced graphene sheets (RGSSs)-bacteria mediated bioimprinted films	$1 \times 10^4 - 1 \times 10^8$	0.7×10^4	[41]
Screen-printed carbon Microarrays	Bacteriophage immobilized over SPE via EDC/NHS	-	10^4	[42]
Gold nanoparticles / free-standing graphene paper electrode	Anti- <i>E. coli</i> O157:H7 antibodies immobilized on paper electrode via biotin-streptavidin system	$1.5 \times 10^2 - 1 \times 10^7$	1.5×10^2	[26]
Screen-printed gold electrode	Endolysin immobilized onto SAM via EDC/NHS	$10^4 - 10^8$	1.1×10^4	[37]
Glassy carbon electrode	Monoclonal antibody physisorbed onto <i>O</i> - carboxymethylchitosan surface-modified Fe ₃ O ₄ nanoparticles	$1.0 \times 10^3 - 10^7$	-	[46]
Screen printed carbon paper (SPC) electrode	Lectin immobilized over electrochemically oxidized carbon surface via EDC/NHS	$10^3 - 10^7$	1.9×10^3	This work

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